Analyses of Mixtures of t,tΔ9,11- and t,tΔ10,12-Linoleic Acids by X-ray Diffraction Patterns and Solidification Points*

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IN a recent investigation of the mechanism of the alkali-isomerization (2) of Δ9,12-linoleic acid it was necessary to identify the conjugated position isomers of linoleic acid present after treatment with alkali. A solid mixture of position isomers, all with trans,trans geometrical configuration about the double bonds, was effected by treatment of the alkali-isomerized acid with iodine. Strong evidence that the resulting product was an equal mixture of t,tΔ9,11- and t,tΔ10,12-linoleic acids was provided by melting point data (2), but a more positive identification of the components was desired.

Isolation of the pure components by fractional crystallization proved impractical since little change in melting point was effected. Since the isomers have identical chromophore groups, their ultraviolet absorption spectra are virtually identical and therefore of no value in the analysis of mixtures. The X-ray diffraction patterns of the crystalline isomers however differ markedly, and the pure isomers can be readily identified by their patterns. Moreover the characteristic pattern of each isomer is obtained from mixtures of the isomers crystallized from the melt or from solvents. Although the analysis of mixtures is somewhat complicated by the solubility of the t,tΔ10,12-isomer in t,tΔ9,11-linoleic acid, the X-ray pattern alone is sufficient to define the composition of mixtures in the range 5 to 75% t,tΔ9,11-linoleic acid. Some other property of the mixture must be measured however if the composition is to be defined over the entire composition range, and for this reason we have measured the solidification point as a function of the composition. In this case the solidification point is a sensitive function of composition, and in conjunction with the X-ray data it defines the composition of any mixture to $\pm$ 3%.

Experimental

Preparation of the Isomers. t,tΔ9,11-Linoleic acid was prepared by the dehydration of ricinelaidic acid according to the procedure of Mangold (1). The isomer was recrystallized from 95% ethanol until the specific absorption coefficient at 231 mμ reached a constant value of 114 in isooctane. The melting point of this material was 54°C., which is the same as that reported by Mangold (1). The iodine number, calculated from measurements of the hydrogen uptake (2) of the methyl ester, was 180.3 (theoretical value, 181.0).

t,tΔ10,12-Linoleic acid was prepared by alkali-isomerization of dehydrated castor oil acids, according to the procedure of von Mikusch (3). The isomer used was recrystallized from a purified petroleum fraction with the boiling range 55-59°C. until the specific absorption coefficient at 231 mμ reached a constant value of 113 in isooctane. The melting point of this material was 56.8°C. von Mikusch (3) reported a melting point of 56-57°C. The iodine number of this material, calculated from measurements of the hydrogen uptake (2) of its methyl ester, was 181.5 (theoretical value, 181.0).

X-ray Technique. Powder patterns of the pure isomers and the mixtures described below were taken with a powder camera of 143.4-mm. diameter. Filtered chromium Kα radiation, λ = 2.2909 Å, was used to obtain maximum dispersion of the diffraction lines. This is particularly important in the analysis of mixtures in order that overlapping of diffractions from the isomers be minimized.

A finely ground sample was extruded from a metal block in the form of a rod 0.5 mm. in diameter. Strong patterns were obtained in four hours by using Eastman Type K film and operating the X-ray tube at 32 kvp. and 18 ma. Background due to air scattering was minimized by filling the camera with hydrogen. Patterns with sharper lines were obtained when the ground sample was pressed on a glass slide and a narrow section of 0.2 mm. thickness cut from it. These samples however required 20-hour exposures, and the background scattering was considerable. Interplanar spacings and the relative intensities of the corresponding diffractions of the pure isomers are reported in Table I.

TABLE I

Comparison of Diffraction Patterns of t,tΔ9,11-Linoleic and t,tΔ10,12-Linoleic Acids With a Pattern of a Fused Mixture Containing 60% t,tΔ10,12-Linoleic Acid and 40% t,tΔ9,11-Linoleic Acid.

t,tΔ9,11-linoleic acid		t,tΔ10,12-linoleic acid		Fused mixture of 60% t,tΔ10,12-linoleic and 40% t,tΔ9,11-linoleic acids	
I obs.	d(Å)	I obs.	d(Å)	I obs.	d(Å)
S+	16.01			M	16.08
		S+	14.68	M	14.68
W	11.99			W=	12.04
		W	11.02	W=	11.00
M	9.60			W+	9.63
		M	8.82	W+	8.81
M-	6.85			W	6.84
		M+	6.39	W	6.28
W	5.99			W=	6.01
		W	5.51	W=	5.49
W-	5.32			W=	5.33
		W+	4.90	W=	4.93
W	4.80	W+	4.78	W-	4.79
W=	4.68	M	4.66	M	4.66
M	4.53	M	4.49	M+	4.49
W	4.35	S	4.35	S	4.35
		W+	4.18	W=	4.28
S++	4.14			S+	4.12
M+	4.08	W+	4.08	M	4.06
W	3.98	W=	3.98	W-	3.99
W	3.88	S+	3.87	S	3.86
W (diff.)	3.73	M+	3.74	M	3.74
M+	3.68	W	3.66	M	3.67
W	3.61	M-	3.59	W+	3.60
W=	3.54				
M	3.48	W+	3.49	W+	3.48

Analysis of Mixtures. It was desired to investigate mixtures prepared by crystallization from a solvent as well as crystallization from a melt. To accomplish this the following procedure was adopted. Mixtures

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weighing approximately 25 mg. were prepared by accurately weighing the pure components and fusing at 60°C. for five minutes in an atmosphere of nitrogen to prevent oxidation. To insure complete mixing the solidified mixture was powdered. A small portion of this mixture was pressed on a glass slide, and a narrow section was cut for the powder camera.

Another portion of the fused and powdered mixture was dissolved in ethyl alcohol and crystallized by distillation of water into the solution. After 16 hours all the material in solution had crystallized on the surface in a platelike mass of oriented crystals. Evaporation of the solution to dryness after removal of the crystalline material left no residue. The crystalline material was pressed on a glass slide, and a narrow section was cut for the powder camera. All the lines on the diffraction patterns of these samples were orders of the long spacing of the two acids because the platelike crystals had oriented parallel to the surface of the solution.

The remaining portion of the fused mixture was used for a solidification point determination. It was packed in melting point tubes, evacuated with a high vacuum pump, sealed off, and stored at 3°.

An estimate of the composition of a mixture can be made from the relative intensities of the diffraction lines of the components. We have measured the intensity of the 8.82 Å line of the t,tΔ10,12-isomer relative to that of the 9.60 Å line of the t,tΔ9,11-isomer on the patterns from fused mixtures. The variation of this quantity, designated IΔ10,12/IΔ9,11, with composition is given in Table III.

Solidification Point Determination. A Fisher-Johns melting apparatus modified to accept a conventional melting point tube was used for the determination of the solidification points of the acids and their mixtures. A horizontal hole drilled through the stage to accommodate a melting point tube intersected a vertical hole, thus permitting observation of the heated sample under a binocular microscope.

A preliminary observation of the solidification point was made, followed by a second determination carried out with carefully controlled rates of heating. The sample was heated at the rate of 0.5° per minute until within 1° of the final equilibrium temperature, then the rate was reduced to .05° per minute. When only a trace of crystalline material remained, the temperature was held constant for 15 minutes to insure equilibrium; by raising or lowering the temperature a fraction of a degree either phase would grow. The solidification point recorded was taken as the temperature at which the last trace of crystalline material remained in equilibrium with the melt.

Different samples taken from the same fused mixtures gave solidification points agreeing within 0.1°. This shows that the method of preparation gave a homogeneous sample and that the sampling procedure used for the X-ray and solidification point measurement was justified. The solidification point did not change with continued remelting and solidification of the sample, indicating that little decomposition, oxidation, or polymerization of the isomer occurs under these conditions. It is therefore unlikely that any similar changes take place in the original fusion of the mixtures. Solidification points obtained according to the method described above are given in Table II.

TABLE II

Solidification Points of Mixtures of t,tΔ9,11-Linoleic and t,t,Δ10,12-Linoleic Acids

Mol-per cent t,tΔ9,11-linoleic acid	Mol-per cent t,tΔ10,12-linoleic acid	Solidification point, °C.
0	100	56.8
5	95	55.7
12	88	54.7
20	80	53.3
32	68	50.8
40	60	49.0
43	57	46.0
50	50	45.1
53	47	45.0
55	45	45.2
60	40	46.1
68	32	49.0
78	22	50.6
80	20	51.0
90	10	52.3
100	0	54.0

Discussion

The interplanar spacings and relative intensities in Table I show that the crystalline isomers can be readily distinguished and identified by the X-ray diffraction data. From these data values of 47.95 Å and 44.09 Å are computed for the long spacings of t,tΔ9,11- and t,tΔ10,12-linoleic acids, respectively.

Although the pure isomers have different crystal structures, as evidenced by their diffraction patterns, it might be expected that mixtures prepared by fusion or crystallization from a solvent would form a continuous series of solid solutions which would be difficult to distinguish by the diffraction method. The experimental data presented in Table III indicate only limited mutual solubility of the isomers. Powder patterns of mixtures containing more than 25% of t,tΔ10,12-linoleic acid showed the characteristic diffraction spectra of each isomer, and the interplanar spacings measured were within the limits of experimental error the same as those of the pure isomers. A comparison of the patterns of the pure isomers with that of a fused mixture containing 60% t,tΔ10,12-linoleic acid and 40% t,tΔ9,11-linoleic acid is given in Table I. Mixtures containing less than 25% of the t,tΔ10,12-isomer gave only the pattern characteristic of pure t,tΔ9,11-linoleic acid. Since there is no evidence for the decomposition of the t,tΔ10,12-isomer in either the X-ray patterns (appearance of new lines due to a third component) or the solidification point data, it is reasonable to assume that the t,tΔ9,11-isomer dissolves about 30% of its weight of the t,tΔ10,12-isomer. t,tΔ9,11-Linoleic acid can be detected in proportions as low as 5% by the diffraction pattern; its solubility in the t,tΔ10,12-isomer must therefore be less than that amount. The X-ray diffraction pattern will thus identify both components

TABLE III

Comparison of the Relative Intensity of the 8.82 Å Diffraction Line of t,tΔ10,12-Linoleic Acid With That of the 9.60 Å Line of t,tΔ9,11-Linoleic Acid in Mixtures

IΔ10,12/IΔ9,11	Mole per cent of t,tΔ10,12-linoleic acid
0	25
0.09	32
.28	40
.48	50
.77	60
2.0	78
3.0	80
4.6	87
7.0	90
16.0	95

of a binary mixture if it contains not less than 25% of the t,tΔ10,12-isomer and not less than 5% of the t,tΔ9,11-isomer. Comparison of intensities of the diffraction lines given in Table III permits an approximate estimate of the composition.

For mixtures containing less than 25% t,tΔ10,12-linoleic acid or less than 5% t,tΔ9,11-linoleic acid, the solidification point must be measured to determine the composition. This assumes that no components other than these isomers are present in the mixture. As shown in Table II, the solidification point is a sensitive function of composition and in conjunction with the X-ray data defines the composition of any mixture to $\pm$ 3%. It is in fact desirable to characterize any mixture by its solidification point since it provides a more precise estimate of the composition than does the diffraction pattern.

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Summary

X-ray diffraction and solidification point data are reported for t,tΔ9,11- and t,tΔ10,12-linoleic acids and their mixtures. The long spacing of the Δ9,11-isomer was 47.95 Å whereas that of the Δ10,12-isomer was 44.09 Å. X-ray diffraction patterns of binary mixtures showed the characteristic pattern of each isomer if the mixture contained not less than 25% of the t,tΔ10,12-isomer or not less than 5% of the t,tΔ9,11-isomer. Outside these composition limits only the pattern of the predominant isomer appeared, and solidification point data were used to define the composition. The solidification point in conjunction with the X-ray data defined the composition of any mixture to $\pm$ 3%.

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